so difficult to study on account of the problem of proper technic that it is not surprising that many incomplete descriptions should have been published. It is to be hoped, however, that the day will come when nematodes will be as thoroughly studied and described as other parasitic worms have been and that their classification and identification will be made more certain.

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AN AMICRONUCLEATE RACE OF PARAMECIUM CAUDATUM

PROBABLY no representative of the Protozoa has received more attention in matters relating to life cycles, reproduction, heredity and cytology than has *Paramecium*. It should be of general interest, therefore, to record the occurrence of a race of *Paramecium caudatum* which appears to be entirely devoid of a micronucleus. The recent studies by Dawson (1) on an amicronucleate race or species of *Oxytricha* add interest to the present discovery.

In the fall of 1914 Doctor M. H. Jacobs of this Laboratory used, in certain heat experiments, some *Paramecium caudatum* derived from a culture which exhibited great viability. During the following January Hance (2), in examining some of the sur-

viving *Paramecia,* found that a few were characterized by the presence of three contractile vacuoles instead of two, the normal number. Several of these animals were isolated and became the progenitors of the multivacuolate race studied by Hance (2). In speaking of the cytology of the race Hance mentions the great difficulty experienced in staining the micronucleus and states that "the depression in the macronucleus where the micronucleus usually lies is frequently visible but it appears quite empty." He decided, however, that there was one micronucleus present.

The greater viability and the slightly larger size of this race as compared with the wild races led to its use for class work. Having occasion, during November, 1919, to filter about four hundred cubic centimeters of classroom culture densely populated by this race of *Paramecium* the writer fixed the animals so obtained in warm Schaudinn's sublimate alcohol and subsequently stained them with Delafield's hæmatoxylin. On examination it was discovered that in none of the individuals could a micronucleus be found. This observation in itself was not conclusive since the seeming absence of the micronucleus might have been due to faulty technique. The same material was stained with borax carmine and the absence of the micronucleus as a staining body was confirmed. Later four different fixatives were used and the material stained with Carmalum and in no case was the micronucleus found. Material was then fixed daily from a series of four cultures for periods ranging from two to four months. Throughout this period the character of the *Paramecia* remained constant in that no multivacuolate animal possessed a micronucleus.

For obtaining pure lines of amicronucleate animals with which to make further observations twenty multivacuolate individuals were isolated. Some of the progeny of each of these were stained in aceto-carmine and in each case the micronucleus was absent. The question as to the identity of the multivacuolate race and the amicronucleate race then arose. The fact that the progeny of twenty multivacuolate individuals showed no micronucleus supported this supposition. A number of slides, made before the discovery of the multivacuolate race by Hance, were found in the Laboratory by Doctor D. H. Wenrich, to whom the writer is greatly indebted for his constant interest, valuable advice, and criticism throughout this preliminary work. Both micronucleate and amicronucleate individuals are to be

found on these slides. Several amicronucleate individuals contain three distended contractile vacuoles. All the micronucleate individuals contain only two. There is reason to believe that these slides were made from the same cultures from which Doctor Jacobs obtained his animals for experimentation. Therefore these slides also indicate the identity of the two races. Apparently both the extra vacuoles and the absence of a micronucleus were characters present before the heat experiments referred to.

Throughout the entire history of the cultures observed the lightly stained, comparatively large, very irregular, and expanded macronucleus is characteristic of the race. Under poor cultural conditions animals with regular nuclei are few in number and these nuclei are usually oval in shape and proportionately larger, more lightly stained than others and often blending with the cytoplasm. Under the same conditions condensations of the chromatin material are of frequent occurrence and consist of three types: (*a*) small or large tongues of chromatin, compact and darkly stained throughout or only around the edges, usually lying in a concavity of the macronucleus, (*b*) small, circular, dense masses of chromatin, usually flattened and near the surface of the macronucleus, (*c*) bar-shaped condensations, many times longer than broad, ranging from very loose aggregations of granules to very compact masses.

In the early part of the work the writer often experienced difficulty in deciding whether or not a micronucleus was present because macronuclear condensations frequently resembled micronuclei. But after observing many specimens they were easily distinguishable since neither condensations, lobes, nor detached portions of the macronucleus possessed the detailed structure typical of a micronucleus. They could always be identified on very careful examination as portions of the macronucleus by the arrangement of the chromatin. It is possible that Hance may have seen macronuclear condensations resembling a micronucleus or small detached portions of the macronucleus which at certain times are rather common.

Other differences between the nuclei of micronucleate and amicronucleate animals were noticed. The macronucleus of the wild, micronucleate races is compact, comparatively small and darkly stained with a distinct concavity for the micronucleus. The nucleus of the amicronucleate race is large, expanded, and lightly staining.

In addition to these characters there are also certain other morphological characters which distinguish the race. The amicronucleate race is larger than the wild ones so far observed. The curve of the buccal groove is slightly greater than that of the micronucleate animals. The posterior tip is slightly bent toward the aboral side and the buccal groove itself is shallower since the sides of the groove are bent outward. All evidences so far indicate that the amicronucleate race has the potentiality of forming from three to seven contractile vacuoles.

Attempts have been made by the writer to induce conjugation. A flourishing culture has been allowed to evaporate to half volume and small mass cultures have been submitted to various experimental conditions but so far the writer has been unable to induce conjugation in this race. Hance (2), however, induced conjugation in the multivacuolate race by the method first mentioned. Amicronucleate animals in conjugation are to be found on the slides (made before Hance's discovery of the extra vacuoles) mentioned above. Hence the race has conjugated in the past and attempts will be made to induce conjugation in the future.

The main question in the future study of this race will be the cytology of the conjugation process. This will require experimental work on methods of inducing conjugation in the race and the study of the conjugants so obtained. The effect, if any, of the absence of the micronucleus on the division process will also be observed.

The maintenance of pure lines and the study of the nuclear changes which proceed in ordinary vegetative existence will also be an important part of the future work. If there is a process of endomixis the same means will provide a basis for the study of that phase.

These two matters are the most interesting from the standpoint of cytology, especially since the work of Calkins and Cull (3) on conjugation, and Erdmann and Woodruff (4) on endomixis, in *Paramecium caudatum* show that the active body is the micronucleus and that the macronucleus breaks down and disappears in both processes.

Is the macronucleus affected by different cultural conditions in any definite way and how does the behavior of the nuclei of the micronucleate race compare with the behavior of the macronucleus in the newly discovered race? The preliminary work done so far indicates that there is a definite relation between

environment and the behavior of the macronucleus and that the macronucleus assumes different shapes and appearances under different cultural conditions.

Is this amicronucleate paramecium able to exist indefinitely without conjugation involving a micronucleus and without reorganization of nuclear material, or is there another type of reorganization in this race? A nuclear reorganization, if present, must evidently be of a different type from that described by Erdmann and Woodruff.

These and similar problems are interesting, not only in themselves, but because *Paramecium* has been studied in great detail by Jennings and others with reference to the occurrence of cytoplasmic variations. The amicronucleate race, however, is important because the variation is one of nuclear structure. The importance and interest of the study is increased by the fact that the micronucleus is usually considered to be an aggregation of generative or hereditary chromatin and the body which supposedly initiates reproductive processes of all types and from which, in sexual reproduction, the new nuclei are formed.

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NOTE ON THE OCCURRENCE OF A PROBABLE SEX-LINKED LETHAL FACTOR IN MAMMALS

THE occurrence of sex-linked lethal factors in *Drosophila* is a matter of common knowledge to most biologists. Since mammals have an essentially similar type of sex determination in so far as their dimorphism of sperm is concerned, it is theoretically possible that sex-linked lethal factors should occur among them.